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Neutralizing Antibody Responses Induced by Varicella-Zoster Virus gE and gB Glycoproteins Following Infection, Reactivation or Immunization

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The purpose of this study was to compare the antibody responses to varicella-zoster virus (VZV) gE and gB after natural VZV infection and after vaccination with live attenuated OKA vaccine in order to determine the relative importance of these proteins as components of a subunit vaccine. Anti-VZV antibody titers determined by IFA were of the same order of magnitude in sera from individuals with a history of varicella and in vaccinated children but higher in individuals given booster vaccination. The titers of anti-gE and anti-gB antibodies were measured by ELISA using recombinant gE or gB as capture antigen. From these experiments, it appears that the ratio of anti-gE to anti-gB antibody is highly variable from one individual to another but relatively stable over a long period of time for a particular individual, even after a zoster episode. Neutralizing antibodies directed against gE or gB were also measured by subtracting the neutralization titers obtained before and after depletion of the specific antibodies on immobilized recombinant gE, gB, or both. This showed that, with respect to neutralization, anti-gE and anti-gB are equally prevalent in vaccinated children and that anti-gE is generally, but not always, predominant over anti-gB in VZV-infected individuals. Finally, antibodies to these two glycoproteins appear to be predominant among the neutralizing antibodies directed to other VZV antigens. ***J. Med. Virol. 53:63–68, 1997.*** © 1997 Wiley-Liss, Inc.

KEY WORDS: varicella-zoster virus; recombinant gE and gB; human humoral immunity; varicella vaccine

INTRODUCTION

Varicella-zoster virus (VZV) causes two diseases, varicella, which is the clinical manifestation of primary infection, and herpes zoster, which occurs predominantly in adults as a consequence of reactivation of latent VZV. Since the sequencing of the whole genome [Davison and Scott, 1986], the understanding of the molecular biology of VZV has made rapid progress. A considerable amount of data had been produced on the immunology of the five VZV glycoproteins (gps) [Arvin et al., 1986; Soike et al., 1987; Brunell et al., 1987; Grose and Litwin, 1988; Diaz et al., 1988; Vafai et al., 1989]. gE, with an Mr of 90–98 kDa, is the most abundant and immunogenic gp and is the predominant gp in VZV-infected human cell membranes [Keller et al., 1984; Montalvo and Grose, 1987; Dubey et al., 1988]. Anti-gE antibody has been demonstrated in subjects with recent primary infection, in subjects vaccinated with the live VZV-Oka vaccine [Harper et al., 1990], and in subjects known to have been infected with VZV in the past [Dubey et al., 1988]. The avidity of IgG antibodies has been shown to increase during the months following primary infection as well as after zoster [Kangro et al., 1991]. Monoclonal antibody (MAb) to VZV gE mediates antibody-dependent cellular cytotoxicity and, in the presence of exogenous complement, also neutralizes virus infectivity in vitro [Grose and Littwin, 1988; Grose, 1989]. Ludovikova et al. [1991] and more recently Kutinova et al. [1996] reported that recombinant vaccinia viruses expressing gE elicited an antibody response in mice capable of neutralizing VZV infectivity in the presence of complement. gB, the second most abundant and immunogenic glycoprotein of the virus is specified by ORF31, located in the U_L region of the VZV genome [Davison and Scott, 1986].

The DNA sequence for gB codes for a polypeptide of 868 amino acid (aa) residues, comprising an eight amino acid signal peptide, the main body of the protein, and a hydrophobic anchor region (aa 699–743), followed by a positively charged C-terminal domain [Keller et al., 1986]. The protein, isolated from the vi-

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TABLE I. Clinical Characteristics of Enrolled Subjects

Identification	Age[a]	Varicella	Zoster	Oka vaccine[b]	Period since last boost[c]
1	30	+	−	+	3 Y
2	42	+	−	+	3 Y
3a	22	+	+	−	6 M
3b	27	+	+	+	1 M
4	35	+	−	−	4 Y
8	37	+	−	−	5 Y
9	63	+	+	−	6 M
11	30	+	?	−	>25 Y
12	63	+	+	−	2 Y
13	67	+	+	−	6 Y
14	Adult	+	−	−	−
15	Adult	+	−	−	?
16	Adult	+	−	−	?
18	63	+	Pneumopathy		1 M
19	Adult	+	−	−	>20 Y
20	82	+	+	−	1 M
21	Adult	+	+	−	1 M
22	Adult	+	−	−	1 M
23	Child	+	−	−	1 W
33	26	+	−	+	1 M
283	40	+	−	−	37 Y
294	Adult	+	+	−	?
503	30	+	−	−	27 Y
169	37	+	−	−	34 Y
523	35	+	−	−	6 Y
697	28	+	−	−	25 Y
Maud	10	+	+	−	4 Y
C1	15–24 M	−	−	+	1 M
C4	15–24 M	−	−	+	1 M
C5	15–24 M	−	−	+	1 M
C6	15–24 M	−	−	+	1 M
C9	15–24 M	−	−	+	1 M
C10	15–24 M	−	−	+	1 M
C12	15–24 M	−	−	+	1 M
C14	15–24 M	−	−	+	1 M

[a]Age at blood sampling.
[b]Seropositive volunteers were subcutaneously inoculated with a single dose of Oka vaccine (Varilrix; SB-Biologicals, Rixensart, Belgium) for a clinical study (unpublished results), which followed standard protocols and was approved by the ethical committee of the Free University of Brussels.
[c]Y, year; M, month; W, week.

rus, consists of a 120–140 kDa heterodimer [Grose et al., 1984], which, upon reduction, dissociates into polypeptides of similar masses (~65 kDa each). When inoculated in animals, purified natural gB has been shown to elicit a humoral response, and both monoclonal and polyclonal antibodies raised against the antigen are able to neutralize viral infectivity in vitro [Grose et al., 1984; Edson et al., 1985; Vafai et al., 1987]. Moreover, a recombinant form of gB was shown to induce neutralizing antibodies [Massaer et al., 1993]. For the present study, purified recombinant gE and gB proteins [Jacquet et al., 1995; Haumont et al., 1996] were used to carry out depletion experiments in order to analyze further the potential of these proteins to stimulate the synthesis of neutralizing antibodies during the course of primary infection or reactivation.

MATERIALS AND METHODS

Serum Samples

Serum samples from 34 subjects were examined in the study (Table I). Fourteen patients (1 child, 13 adults) had a history of varicella only (no vaccine), generally during childhood, although two (Nos. 4 and 8) were infected by their children 4 and 3 years, respectively, prior to blood sampling. Three adults (Nos. 1, 2, 33) had a history of varicella and were boosted with a single dose of live VZV-Oka vaccine (Varilrix; SB-Biologicals, Rixensart) three years (Nos. 1 and 2) or 1 month (No. 33) before collecting the blood samples.

Seven subjects (Nos. 3a, 9, 12, 13, 20, 21, and 294) had a history of both varicella and zoster. Subjects 12 and 13 represent the same individual bled 4 years apart. Subject 3b corresponds to patient 3a, who was boosted with VZV-Oka vaccine 4 years after a zoster episode.

The last group (C1–C14) comprised 8 children who were seronegative for antibody to VZV when inoculated with a single dose of live varicella vaccine (Varilrix) at the age of 15–24 months, in the course of a clinical trial to be reported elsewhere. The serum samples were taken 42 days postvaccination.

Titration of Anti-VZV Antibodies

Anti-VZV antibodies were assayed using a commercial indirect immunofluorescent antibody (IFA) test kit (Virgo, Columbia, MD), used as recommended by the

manufacturer. Briefly, eight-well slides of VZV-infected and uninfected fixed cells were incubated with serial dilutions of the sera, then rinsed and incubated with fluorescein isothiocyanate (FITC)-conjugated goat antihuman IgG. Slides were observed at a magnification of ×200. Antibody titers are expressed as the reciprocal of the last dilution giving a positive signal.

Titration of Anti-gE and Anti-gB Antibodies

The antibody titers were measured by the reactivity of serial dilutions of the sera with recombinant gE or gB. Immunoplates (96 wells; Nunc) were coated with 500 ng of recombinant gE or gB prepared and purified as described by Haumont et al. [1996] and Jacquet et al. [1995]. Immunoplates were washed five times with 100 μl/well of TBS-T (Tris 50 mM, NaCl 150 mM, Tween 80 0.1%, pH 7.5) and saturated for 1 hr at 37°C in the same buffer supplemented with 1% bovine serum albumin (BSA). Sera (100 μl of serial dilutions in TBS-T-BSA) were incubated with the antigen for 1 hr at 37°C. Plates were washed (five times with 100 μl TBS-T), and the reactive antibodies were detected with antihuman phosphatase-conjugated goat antibodies (1/7,500 dilution in TBS-T-BSA; Promega, Leyden, The Netherlands). Alkaline phosphatase activity was assayed using paranitrophenylphosphate (Sigma, ST. Louis, MO) in diethanolamine buffer (pH 9.8) as substrate. Titers are expressed as the reciprocal of the dilution giving one-half the maximal OD at 415 nm (Biorad Novapath Microplate Reader).

Neutralization Assay

Assay of antibody-mediated neutralization of VZV infectivity in vitro was undertaken as follows. Aliquots of 10^4 pfu of lyophilized cell-free Webster VZV were rehydrated in 1 ml H_2O and diluted to 4×10^2 pfu/ml in VZV buffer [phosphate-buffered saline (PBS), saccharose 5%, glutamate 1%, fetal bovine serum (FBS) 10%, pH 7.1]. Virus (100 μl) was incubated for 1 hr at 37°C with 100 μl heat-inactivated serum serially diluted in VZV buffer. Guinea pig serum (2 μl; Gibco, Grand Island, NY) complement was added to each well. Virus and serum were incubated for 2 hr at 37°C and plated onto a confluent MRC-5 monolayer (24-well microplates; Nunc) at room temperature in the dark. Two hours later, 800 μl of culture medium (RPMI plus 2% FCS) was added. Seven days later, the medium was removed, and cells were fixed and stained with Coomassie blue solution (Coomassie blue 0.5%, methanol 45%, acetic acid 10%) for 10 min. Plates were washed with distilled water, and lysis plaques were counted. Each dilution was tested in duplicate. The neutralizing titer is expressed as the reciprocal of the serum dilution resulting in 50% neutralization as estimated from a graphical interpolation between the two closest points.

Coupling of gE and gB to Sepharose

gE and gB were coupled to activated Sepharose CH4B (Pharmacia, Uppsala, Sweden). One microgram of gE or 1 mg of gB, dialyzed against the coupling buffer ($NaHCO_3$ 0.1 M, NaCl 0.5 M, pH 8.0), was incubated for 4 hr at 4°C under gentle agitation with 3 ml of CH4B gel in 9 ml of coupling buffer. The supernatant of the gel was removed and assayed for uncoupled proteins. Under these conditions, the coupling efficiency was above 97% as determined by ELISA before and after coupling. After the coupling step, the reactive groups were blocked with Tris HCl 0.1 M, pH 8.0, and the gel was washed by three cycles of alternating pH 4 and pH 8 buffers as recommended by the manufacturer. Finally, the gel was equilibrated in PBS (pH 7.5) and stored at 4°C.

TABLE II. Anti-gE, Anti-gB, and Neutralization Titers of Individuals With a Past History of Varicella Only[a]

Identification	IFA	Anti-gE titer	Anti-gB titer	Neutralization titer
4	120	409	292	nd
8	16	nd	nd	nd
11	64	nd	nd	60
14	n.t.	900	nd	30
15	120	287	228	30
16	n.t.	230	100	30
18	16	109	201	60
19	128	100	850	480
22	256	360	nd	n.t.
23	n.t.	380	270	120
169	128	399	631	120
283	256	600	450	120
503	512	600	450	120
697	128	215	577	240

[a]IgG antibody titers assayed by IFA are expressed as the reciprocal of the end point dilution as described in Materials and Methods. Anti-gE and anti-gB antibody titers are expressed as the reciprocal of the dilution giving a signal of 50% of the maximal value for the serum concerned. Titers are the means of duplicates. Neutralization titers are expressed as the reciprocal of the dilutions leading to 50% of the maximal number of plaques. nd, Not detectable; nt, Not tested.

Anti-gE and Anti-gB Depletion in the Protein Solution

Aliquots of 100 μl of gE or gB gels were incubated overnight at 4°C with 100 μl of serum and 400 μl of PBS. The supernatant of the gel was harvested and pooled with twice 250 μl of PBS used to rinse the gel. After depletion, 100 μl of serum was recovered in a total volume of 1 ml. Control depletion with BSA coupled to CH4B, which was carried out on each serum, always resulted in less than a 10% reduction of anti-gE or anti-gB titers. After the specific depletions, the titers of anti-gE or anti-gB were always below the detectable level, indistinguishable from a control nonimmune serum.

RESULTS

Anti-VZV Antibodies

Whole anti-VZV antibodies were assayed by an indirect immunofluorescent test. Anti-VZV titers in children who were immunized with Oka vaccine (GMT 197.4 ± 91.8; n = 8) were approximately twofold higher than the titers obtained in adults who had a past history of varicella only (GMT 104.7 ± 83.4; n = 11; Tables II, III). The titers in subjects who had a history of varicella and zoster or booster with Oka vaccination (Table

TABLE III. Anti-gE, Anti-gB, and Neutralization Titers in Children Vaccinated With Live Oka Vaccine[a]

Identification	Depletion of	Anti-gE titer	Anti-gB titer	Neutralization titer	IFA
C1	None	281	215	120	256
	Anti-gE	nd	244	60	
	Anti-gB	242	nd	60	
C4	None	512	389	240	512
	Anti-gE	nd	447	160	
	Anti-gB	430	nd	120	
C5	None	361	507	120	128
	Anti-gE	nd	468	80	
	Anti-gB	291	nd	40	
C6	None	784	529	240	128
	Anti-gE	nd	463	80	
	Anti-gB	711	nd	60	
C9	None	409	922	120	256
	Anti-gE	nd	742	60	
	Anti-gB	218	nd	40	
C10	None	609	308	120	256
	Anti-gE	nd	335	40	
	Anti-gB	566	nd	60	
C12	None	308	483	120	128
	Anti-gE	nd	454	120	
	Anti-gB	235	nd	40	
C14	None	675	605	240	128
	Anti-gE	nd	593	120	
	Anti-gB	623	nd	60	

[a]Legend is the same as for Table II. Children's sera were obtained from a clinical study conducted by SB-Biologicals.
Serum depletion was performed as described in Materials and Methods.

IV) were significantly higher ($P = 0.029$ and 0.055, respectively, using Student's t-test), the GMT being >445.7 ± 347.8 (n = 10). The highest titers were obtained with zoster patients (Nos. 9, 20, and 294), although two other subjects showed low titers (Nos. 21 and 12:13, corresponding to the same individual 2 and 6 years after zoster).

Anti-gE, Anti-gB, and Neutralization Assays

Anti-gE and anti-gB antibodies were assayed by ELISA as described in Materials and Methods using recombinant gE and gB as capture antigens. Neutralizing antibodies were assayed using cell free VZV of the Webster strain.

Table II shows the results obtained with subjects who had a history of varicella only. Antibody as well as neutralization titers were very low in these individuals, including the two adults (Nos. 4 and 8) who were infected only 4 and 5 years before testing.

Six subjects had a significant neutralizing titer (above 60), but this was never higher than 480 (No. 19). In this group, the neutralizing titer was correlated better with the anti-gB titer than the anti-gE titer (correlation coefficients of 0.86 and 0.41, respectively). The highest neutralizing titers were observed for subjects 19 and 697, who had high anti-gB and low anti-gE titers, whereas a low neutralizing titer was observed in subject 14, who had a high anti-gE and a low anti-gB titer.

Table III shows the results obtained from vaccinated children. All the sera had significant levels of antibodies to gE and gB and neutralizing activity. The titers were not significantly different compared to those in adults who had a past history of varicella (anti-gE GMT 462.9 vs. 319.2, $P = 0.251$; anti-gB GMT 455.7 vs. 343.2, $P = 0.406$; neutralizing GMT 155.6 vs. 87.6, $P = 0.431$).

Table IV shows the results obtained with subjects who had a past history of varicella followed by either zoster or vaccination, or both. All the sera had significant levels of antibodies to gE and gB and neutralizing activity. There was no obvious correlation between antibody and neutralizing titers. Two adults who were tested twice deserve particular attention. Subject 3a was first tested 6 months after a zoster episode and then had a moderate level of gE/gB antibodies as well as neutralizing activity. Five years later (No. 3b), he was boosted with a single dose of Oka vaccine, and, although both anti-gE and anti-gB were found to be raised 1 month after vaccination, the neutralizing titer remained unchanged. The second adult was a woman of age 61 years at the onset of zoster. She was bled 2 years (No. 12) and 6 years (No. 13) later. Between the two sera, her antibody titer increased while her neutralizing titer decreased.

Serum Depletion of Anti-gE or Anti-gB

Sera were depleted of anti-gE or anti-gB antibody with recombinant gE or gB coupled to Sepharose, as described in Materials and Methods. As a control, the depletion protocol was undertaken with Sepharose-coupled BSA only. Antibody titers never decreased by

TABLE IV. Antibodies From Sera of Subjects Who Had a Past History of Varicella and Zoster or Oka Vaccination: Depletion of Anti-gE or Anti-gB[a]

Ident.	IFA	Depletion of	Anti-gE titer	Anti-gB titer	Neutralization titer
1	512	None	200	3,950	1,920
		Anti-gE	nd	3,500	480
		Anti-gB	180	nd	480
2	64	None	165	135	240
		Anti-gE	nd	140	60
		Anti-gB	175	nd	240
3a	512	None	160	350	480
		Anti-gE	nd	380	240
		Anti-gB	140	nd	120
		Anti-gE anti-gB	nd	nd	120
3b	>512	None	640	1,800	480
		Anti-gE	nd	1,950	480
		Anti-gB	650	nd	120
9	>512	None	3,200	650	3,840
		Anti-gE	nd	630	1,280
		Anti-gB	2,500	nd	3,840
12	256	None	340	320	480
		Anti-gE	nd	300	240
		Anti-gB	320	nd	240
		Anti-gE anti-gB	nd	nd	60
13	256	None	2,400	1,800	120
20	1024	None	2,500	900	3,840
		Anti-gE	nd	880	1,920
		Anti-gB	2,400	nd	3,840
		Anti-gE anti-gB	nd	nd	480
21	n.t.	None	400	200	n.t.
33	512	None	520	1000	60
		Anti-gE	nd	880	nd
		Anti-gB	380	nd	nd
294	2048	None	2,700	1,060	7,680
		Anti-gE	nd	1,000	1,920
		Anti-gB	2,700	nd	7,680
Maud	n.t.	None	600	260	960
		Anti-gE	nd	220	480
		Anti-gB	630	nd	640

[a]Legend is the same as for Table II. Serum depletion was performed as described in Materials and Methods.

more than 10% under these conditions. Conversely, the specific depletions always resulted in undetectable levels of antibodies.

Two sets of depletion experiments were performed. First, depletion of antibodies from sera from the children immunized with the Oka vaccine (Table III) suggested that, in this case, anti-gB contributed to neutralization somewhat more than anti-gE. Indeed the neutralizing GMTs dropped from 155.6 to 56.2 and 82.4, respectively. Second, the depletion of antibodies from sera from individuals who had experienced varicella followed by zoster or Oka vaccination (Table IV) showed that in four cases (Nos. 2, 9, 20, and 294), the anti-gB antibodies did not appear to contribute significantly to neutralization, whereas for three subjects (1, 12, and 33) anti-gE and anti-gB seemed to contribute equally. In one individual (3a and 3b) who was tested before and after vaccination, anti-gB antibodies seemed to contribute to neutralization more than anti-gE.

The sera from three subjects were depleted of both anti-gE and anti-gB antibodies through consecutive adsorption on the respective columns. In two cases (Nos. 12 and 20), the effect proved additive, whereas in one case (No. 3a) this effect was not observed. Taken together, these results suggest that anti-gE and anti-gB are both important for neutralization in a ratio that may be highly variable from one individual to another.

DISCUSSION

It is known that VZV glycoproteins induce strong humoral responses both in naturally infected individuals and in varicella vaccine recipients [Dubey et al., 1988; Harper et al., 1990; Watson et al., 1994]. Although antibodies to the three major glycoproteins have been assayed [Brunell et al. 1987; LaRussa et al 1990], the relative contribution of the two main glycoproteins, gE and gB, to neutralizing antibody titers has never been established. To measure this effect, we used recombinant gE and gB to deplete sera from various groups of individuals of the respective antibodies. Absorption with the recombinant proteins depleted the sera by >95% of the antibody to the recombinant gE or gB.

Thirty-four sera were tested. Fourteen sera were from individuals who had a past history of varicella only, 8 were from children who were vaccinated with the VZV-OKA strain, and 12 were from patients who had a history of varicella and zoster or varicella vaccination. Antibody titers by IFA were of the same order

for patients who had a history of varicella and for children who were vaccinated with the Oka vaccine, but, as expected, the titers were significantly higher in individuals whose responses had been boosted either by an episode of zoster or by vaccination. These results were in the range of those observed by others.

Specific antibody titers against gB and gE, as well as neutralizing titers, also were not significantly different in children who were immunized compared to those in adults who reported varicella in their childhood. Depletion experiments showed that the contribution of anti-gB antibodies to neutralization may be more important than the contribution of anti-gE following vaccination.

Adults who had experienced varicella in childhood and were boosted either by a zoster episode or by vaccination had significantly higher titers. Depletion experiments showed that anti-gE contributes generally, but not always, more than anti-gB to neutralization. Depletion of both anti-gE and anti-gB was performed on the sera from three patients and reduced the neutralizing titers, showing that these two antigens are highly dominant in this respect. The results suggest that both gE and gB may be necessary and equally important components for a future subunit vaccine against VZV infection.

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